Zinc copper levels in patients with primary hyperparathyroidism

Primer hiperparatiroidi hastalarında çinko bakır düzeyleri

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SUMMARY

Objective: Primary hyperparathyroidism (PHPTH) is an endocrine disorder with hypercalcemia due to increased parathormone (PTH) release. This study aims to investigate the relationship between PHPTH and serum zinc and copper levels.
Method: 35 patients diagnosed with PHPT and 30 healthy control cases were included in the study. Serum zinc and copper levels were measured in all patients.
Results: There was no difference between the groups in terms of age and gender. It was detected that serum Zn levels were significantly lower and serum Cu levels were higher in the PHPTH group compared to the control group (PHPTH Zn: 85.8±24.9 µ/dL, Cu: 81.2±13.8 µ/dL and Control Zn: 103.3±21.3 µ/dL, Cu: 72.6±13.3 µ/dL p = 0.004, p= 0.013, respectively). There was a weak positive correlation between serum Cu levels and PTH levels.
Conclusions: Serum zinc and copper levels should be considered in the clarification of the pathogenesis of PHPTH and in treatment planning.
Keywords: Primary hyperparathyroidism, zinc, copper

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ÖZET

Amaç: Primer hiperparatiroidi (PHPTH), artmış parathormon (PTH) salınımına bağlı hiperkalsemi ile seyreden endokrin bozukluktur. Bu çalışma, PHPTH ile serum çinko, bakır düzeyleri arasındaki ilişkiyi araştırmayı amaçlamaktadır.
Yöntem: Çalışmaya PHPT tanısı konmuş 35 hasta ve 30 sağlıklı kontrol olgusu dahil edildi. Tüm hastalarda serum çinko ve bakır düzeyleri ölçüldü.
Bulgular: Gruplar arasında yaş ve cinsiyet açısından fark yoktu. PHPTH grubunda serum Zn seviyeleri kontrol grubu ile karşılaştırıldığında önemli ölçüde düşük, serum Cu seviyeleri ise yüksek saptandı (sırasıyla PHPTH Zn: 85.8±24.9 µ/dL, Cu: 81.2±13.8 µ/dL ve Kontrol Zn: 103.3±21.3 µ/dL, Cu: 72.6±13.3 µ/dL p = 0.004, p= 0.013). Serum Cu seviyesi ile PTH seviyesi arasında pozitif zayıf korelasyon vardı.
Sonuç: Serum çinko ve bakır düzeyleri, PHPTH patogenezinin aydınlatılmasında ve tedavi planlanmasında göz önünde bulundurulmalıdır.
Anahtar sözcükler: Primer hiperparatiroidi, çinko, bakır

INTRODUCTION

Primary hyperparathyroidism (PHPT), which is the most important cause of hypercalcemia, usually occurs with hypercalcemia and increased parathyroid hormone (PTH) levels. It is caused by excessive synthesis and secretion of PTH by one or more of the four parathyroid glands [1]. PTH in bone; regulates osteoclast formation and increases serum Ca+ level. Moreover, PTH stimulates 1-α hydroxylation of the kidney [2]. 1.25 D3 increases Ca+ absorption by increasing Ca+ cotransport expression in the mucosa of the small intestine and duodenum. Therefore, PTH and 1.25 D3 are important factors in the regulation of bone remodelling. Zinc (Zn) is also important in bone remodeling. Zn is abundant in bone tissue and 30% of its total is in the bone. Zn stimulates osteoblastic bone formation and mineralization. It inhibits osteoclastic bone resorption by inhibiting osteoclasts-like cell formation from the bone marrow. Additionally, in-vitro mature osteoclast stimulates apoptotic cell death [3]. It has been shown that zinc deficiency disrupts bone growth, development and preservation of bone strength. Copper also plays an important role in the regulation of bone growth and skeletal development. Copper induces the formation of the lysine cruciate ligaments in collagen and elastin via lysyl oxidase activation. As a cofactor of antioxidant enzymes, it removes free radicals of bone that cause osteoclast activation [4]. In addition, copper directly inhibits osteoclastic bone resorption [5]. Copper, increases the total bone strength and helps maintain the optimum state of bone quality [6].

There haven't been any studies conducted on patients with primary hyperparathyroidism related to serum zinc and copper levels which have been studied in many areas in the recent years. Therefore, we aimed to evaluate serum zinc and copper levels in patients with Primary Hyperparathyroidism. We studied the relationship between serum Vit D, Ca, Magnesium (Mg), Phosphate (P) and zinc, copper levels.

MATERIAL AND METHODS

This cross-sectional study was conducted by Adana Numune Training and Research Hospital, Department of Internal Medicine and Kahramanmaraş Sütcü Imam University Faculty of Medicine Department of Biochemistry. The study protocol was approved by The Local Ethics Comittee (Date: 07 11. 2018, Descision no:5) Written consent was received from all participants.

Study Population

35 patients diagnosed with PHPT in Adana City Hospital between the years of 2012-2017 and 30 healthy control cases were included in the study. Patients diagnosed with multiple endocrine neoplasms, parathyroid cancer, thyroid cancer, hyperparathyroidism-jaw tumor syndrome (HPT-JT), familial hypocalciuric hypercalcemia and patients who were admitted to the hospital with a symptom related to calcium and vitamin D metabolism at least 2 weeks before were excluded from the study. Primary hyperparathyroidism was diagnosed in the existence of the two parameters below as; serum calcium being above 0.1 mg/dl, serum iPTH level being higher, higher/normal or normal at a level incompatible with hypercalcemia compared to the laboratory normal [7].

In a study examining the zinc levels in patients with secondary hyperparathyroidism [8], there was a 95.4% difference in zinc levels detected between the secondary hyperparathyroidism groups and the control group. Based on the aforementioned study, power analysis with a test power of 0.95 by accepting the error as 0.05 required the inclusion of 29 patients in each group taking into account the ratio of at least 95.4% in terms of zinc levels.

Serum Zinc-Copper Measurements and Evaluated Serum Parameters

Fasting blood samples were taken from healthy controls and patients in 10 cc biochemistry gel tubes. These tubes were centrifuged for 5 minutes at 4000 g. The acquired serum samples were frozen at 80° C until analysis. Following 1/4 dilution with 5% glycerol for zinc level detection and 1/2 dilution with 10% glycerol for copper level detection, the results were calculated in μg/dL determining via flame spectrophotometry in the Perkin Elmer Analyst 800 model atomic absorption spectrometry device. Concurrent serum Ca^{+2}, Mg^{+2}, Vit D levels from the day blood collection were acquired from the automation system.

Statistical Analysis

The data were analyzed using the SPSS 20.0 program for Windows (SPSS, Inc., Chicago, IL, USA). For statistical evaluation, the compatibility of the data with normal distribution was analyzed with the Kolmogorov-Smirnov test. Two sample independent t test was performed for the data compatible with normal distribution and Mann-Whitney U test was performed for the data not showing normal distribution in the two group comparisons for the patient and control groups. Statistical parameters were expressed as Mean±SD for data with normal distribution and as median

(Min-Max) for variables without normal distribution. The relationship between the variables was analyzed with spearman correlation test. Roc Curves were used to determine the differentiation of serum Zn and Cu^{+2} levels and cut-off values were determined. Statistical significance was accepted as p<0.05.

RESULTS

General characteristics of the study population

A total of 65 individuals were included. There was no difference between the groups in terms of age and gender (p>0.05). Demographic data are shown in Table 1. Serum Zn levels were significantly lower and serum Cu^{+2} levels were higher in the PHPTH group compared to the control group (PHPTH Zn: 85.8±24.9 µ/dL, Cu^{+2}: 81.2±13.8 µ/dL and Control Zn: 103.3±21.3 µ/dL, Cu^{+2}: 72.6±13.3 µ/dL p = 0.004, p= 0.013, respectively). (Figure 1) Correlations between serum Zn and Cu^{+2} levels and other laboratory results were examined (Table 2). There was a weak positive correlation between serum Cu^{+2} level and PTH level (r = 0.246; p = 0.049). (Figure 2)

ROC curve was drawn for serum Zn and Cu^{+2}. Low values showed the presence of PHPTH with 93.6 cut-off point, 0.714 sensitivity and 0.667 specificity for Zn. High values showed the presence of PHPTH with 74.3 cut-off point, 0.714 sensitivity and 0.567 specificity for Cu. (Figure 3)

Table 1: The laboratory and demographic characteristics of study population

	Primary Hyperparathyroidism (n=35)	Control (n= 30)	*P* value
Gender (E, K)	E:4 / K:31	E:8/K:22	0.199
Age (years)	54.4±13.8	49.0±7.4	0.060
PTH (ng/L)	**186 (48-1344)**	**50 (22-105)**	**0.000***
Vit D (pg/mL)	16.6 (3.4-51.3)	19.5 (7.3-30.9)	0.175
Ca (mg/dL)	**11.2±0.6**	**9.5±0.5**	**0.000***
P (mg/dL)	**2.8±0.5**	**3.3±0.5**	**0.000***
Mg (mg/dL)	**1.9 (1.6-2.3)**	**2.1 (1.9-2.2)**	**0.010***
ALP (U/L)	**93 (55-326)**	**60 (44-143)**	**0.000***
Zn (µ/dL)	**85.8±24.9**	**103.3±21.3**	**0.004***
Cu^{+2} (µ/dL)	**81.2±13.8**	**72.6±13.3**	**0.013***

*p <0.05 significant

¥ p value is based on two independent samples t test and Mann-Whitney U Testi.

Values were expressed as mean+standard deviation and median (minimum-maximum).

PTH; Parathormone, Vit D; Vitamin D, Ca; Calcium, P; Phosphorus, Mg; Magnesium, ALP; Alkaline phosphatase, Zn; Zinc, Cu^{+2}; Copper.

Table 2: Correlation analysis of study parameters

		PTH	Vit D	Ca^{+2}	P	Mg^{+2}	ALP	Dexa	Zn	Cu^{+2}
PTH	r value	1	-0.328	0.703	-0.395	-0.258	0.453	0.002	-0.180	0.246
	p value		0.010*	0.000*	0.001*	0.039*	0.000*	0.993	0.152	0.049*
D vit	r value	-0.328	1	-0.104	0.259	0.163	0.096	0.174	0.136	-0.041
	p value	0.010*		0.427	0.044*	0.214	0.486	0.326	0.296	0.752
Ca^{+2}	r value	0.703	-0.104	1	-0.493	-0.270	0.486	-0.163	-0.204	0.187
	p value	0.000*	0.427		0.000*	0.031*	0.000*	0.358	0.104	0.136
P	r value	-0.395	0.259	0.493	1	0.242	-0.159	0.357	0.109	-.058
	p value	0.001*	0.044*	0.000*		0.054	0.242	0.038*	0.389	0.648
Mg^{+2}	r value	-0.258	0.163	-0.270	0.242	1	0.200	-0.055	0.226	-0.168
	p value	0.039*	0.214	0.031*	0.054		0.140	0.759	0.073	0.184
ALP	r value	0.453	0.096	0.486	-0.159	0.200	1	0.170	-0.218	0.260
	p value	0.000*	0.486	0.000*	0.242	0.140		0.344	0.107	0.053
Dexa	r value	0.002	0.174	-0.163	0.357	-0.055	0.170	1	0.068	-0.070
	p value	0.993	0.326	0.358	0.038*	0.759	0.344		0.704	0.682
Zn	r value	-0.180	0.136	-0.204	0.109	0.226	-0.218	0.068	1	-0.218
	p value	0.152	0.296	0.104	0.389	0.073	0.107	0.704		0.082
Cu^{+2}	r value	0.246	0.187	0.187	-.058	-0.168	0.260	-0.070	-0.218	1
	p value	0.049*	0.136	0.136	0.648	0.184	0.053	0.682	0.082	

PTH; Parathormone, Vit D; Vitamin D, Ca^{+2}; Calcium, P; Phosphorus, Mg^{+2}; Magnesium, ALP; Alkaline phosphatase, Zn; Zinc, Cu^{+2}; Copper. DEXA: Dual energy X- ray Absorptiometry
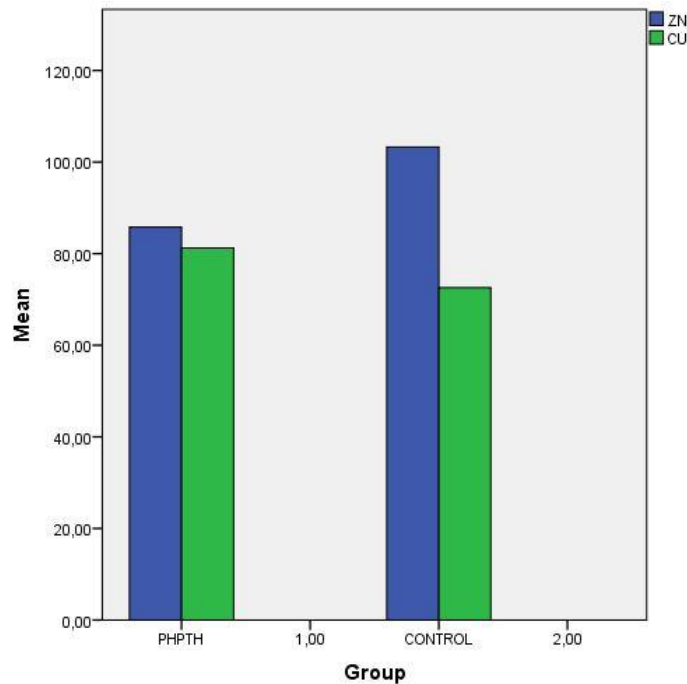

Figure 1: Serum Zn and Cu^{+2} concentrations of primary hyperparathyroidism and control groups
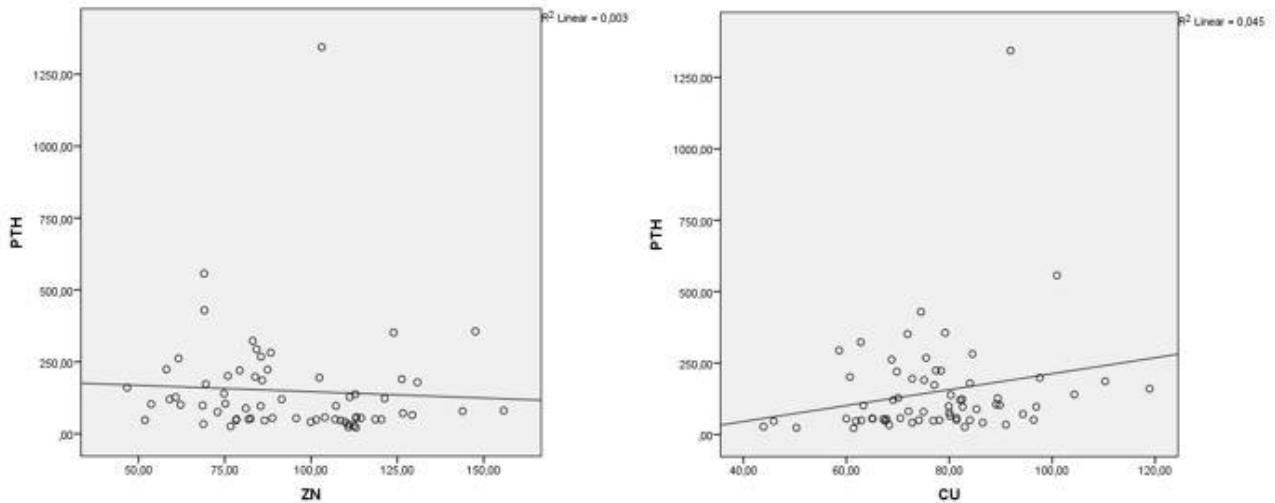
Figure 2: Serum PTH levels were significantly positively correlated with Cu^{+2}.
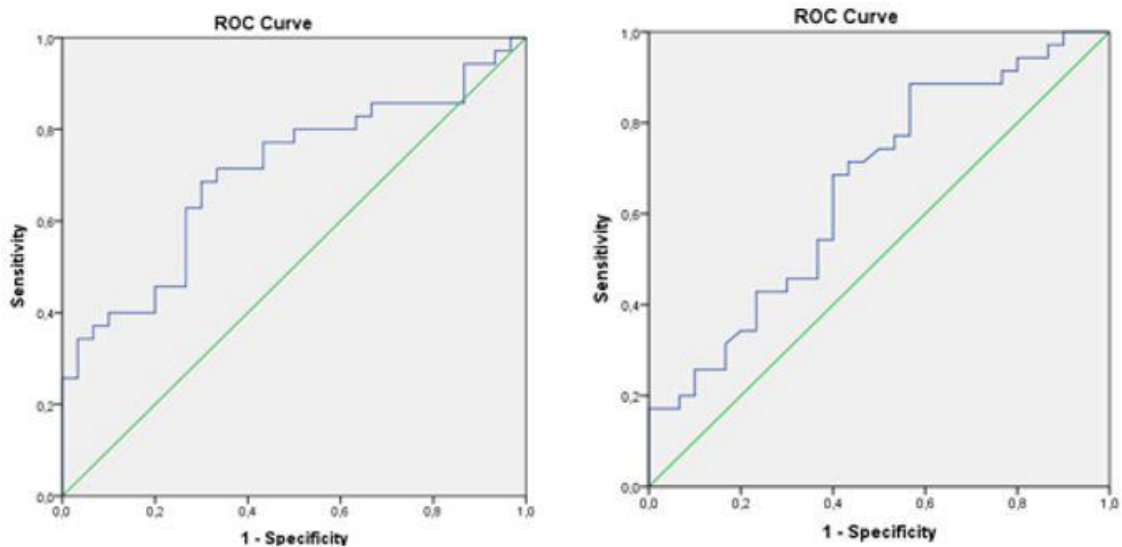

Figure 3: ROC curve for serum Zn and Cu^{+2} (respectively)

DISCUSSION

In this study, we detected lower serum zinc and higher serum copper levels in patients with primary hyperparathyroidism compared to the healthy control group. There was also a weak positive correlation between serum Cu^{+2} levels and PTH levels.

Zinc is a cofactor that participates in cell growth through the activation of enzymes involved in DNA, RNA and protein synthesis, and therefore has various biological functions including bone metabolism in the body. It has been shown that zinc increases osteoblastic activity and collagen synthesis, and also reduces osteoclat-mediated bone resorption, thereby shifting the cycle balance towards an anabolic profile [9]. Additionally, it has been suggested that zinc deficiency can indirectly affect bone health by reducing the calcium absorption in the intestine and increasing the circulating parathyroid hormone (PTH), which activates the bone cycle [10]. The relationship between serum PTH and trace element in children with chronic kidney disease (CKD) undergoing hemodialysis was studied and contrary to zinc deficiency increasing PTH Zinc levels, zinc levels were found low in the patient group with CKD and high PTH. Moreover, a negative correlation was shown between serum Zn and PTH levels [11]. It was suggested that hypozincemia may be caused by the chronic uremia observed in CKD increasing urinary Zn excretion and decreasing intestinal Zn

absorption and that the increased PTH in uremia may have played a role in the pathogenesis and it was shown that PTH increases extra renal zinc excretion and liver Zn absorption [12]. In another study conducted on hemodialysis patients, Zn levels were found lower in the patient group compared to the control group, but a correlation wasn't shown between Zn and PTH levels [13]. Copper serves as a cofactor for various enzymes, especially for metalloenzymes involved in reducing molecular oxygen through oxygenase activities. Copper plays an important role in the regulation of skeletal bone growth and development. This element induces the formation of the lysine cruciate ligaments in collagen and elastin through the activation of lysis oxidase. It shows its antioxidant role in oxidative stress by eliminating free radicals in the bone that cause osteoclast activation as a cofactor of antioxidant enzymes [4]. Moreover, copper directly prevents osteoclastic bone resorption [5]. Copper, all together, increases bone strength and helps maintain optimum bone quality.

Although many studies have been conducted on zinc levels in secondary hyperparathyroidism, in the first and only study on zinc copper levels in patients with primary hyperparathyroidism, urine zinc and copper excretions were found higher in patients with primary hyperparathyroidism and while there was no difference in the serum Zn levels, serum copper levels were found higher compared to the healthy control group [14]. In this study, serum Zn levels were low and Cu^{+2} levels were high in patients with primary hyperparathyroidism. The underlying mechanism of this effect is unknown. However, it may include the direct effect of PTH on zinc's synthesis, release, metabolism, clearance and/or effect at a cellular level.

In addition, Zinc shows antioxidant properties by inhibiting the production of reactive oxygen and nitrogen species, taking a structural role in antioxidant proteins and acting as a coenzyme in antioxidant enzymes [15,16,17]. Studies have shown that excessive copper exposure may cause excessive production of ROS (reactive oxygen species) and oxidative stress triggered by decreased antioxidant function [18,19,20]. It was suggested that low zinc and high copper levels in the patient group may have increased oxidative stress, suggesting that this may play a role in the development of PHPTH.

In conclusion, in patients with PHPTH, the negative correlation between high PTH and hypozincemia may indicate a causal relationship. However, it may be considered that Primary hyperparathyroidism is also increased oxidative stress and therefore may cause high serum Cu^{+2} levels [15]. In order to clarify this relationship, it is recommended for further studies to be conducted on a large number of patients with PHPTH.

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